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TITLE: An Epidemiologic Study of Genetic Variation in Hormonal Pathways in Relation to the Effect of Hormone Replacement Therapy on Breast Cancer Risk

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14. ABSTRACT
CHT use has been demonstrated to confer an increased risk of breast cancer. Genetic variation in hormonal pathways may modify the effect of CHT on breast cancer risk. Using 1237 cases and 1015 controls from two population-based case-control studies of breast cancer, we investigated the effect of genetic variation in 7 genes within the progesterone pathway using a tagSNP and functional SNP approach and 5 genes within the catechol estrogen pathway. Within single gene analyses we observed breast cancer risk to be modestly associated with one SNPs in each GSTP1 (rs1695: OR = 1.4 [95% CI: 1.02-1.9] for carriers of A allele); CYP1B1 (rs1056827: OR = 1.7]95% CI:1.2-2.5] for T homozygotes); SRD5A1 (rs248793: OR=1.2 [95% CI: 1.02-1.5] for G homozygotes) and PGR (rs492457: OR=1.5 [95% CI: 1.01-2.1] for carriers of the A allele). We found that the breast cancer risk associated with SNPs was particularly strong in long-term CHT users. In a multi-gene model including two genes with single gene effects within the estrogen pathway (CYP1B1*2 and GSTP1), breast cancer risk was 1.6 (95% CI: 1.03-2.4) times higher for carriers of 1 high risk genotype and 2.8 (95% CI: 1.5-5.3) times higher for women with 2 high risk genotypes compared to women with 0 high risk genotypes. The impact of high risk genotypes was stronger in long-term CHT users, particularly in long-term, current CHT users (OR=5.6 [95% CI: 1-5-20.6]). These results suggest that breast cancer risk among CHT users is modified by variation in genes within hormonal pathways.

15. SUBJECT TERMS
Genetic polymorphisms, epidemiology, exogenous risk factors, gene-environment interactions, hormonal pathway, estrogen, progesterone, tagSNP

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Introduction

This document describes the work completed during the second year of the pre-doctoral training grant for the Breast Cancer Research Program. The majority of tasks outlined in the statement of work have been completed.

Body

All tasks outlined for months 1-24 in the statement of work have been completed (Appendix Table 1.) Firstly, the genotyping of 71 SNPs in 7 genes within the progesterone metabolism and receptor pathway among 2351 women (for my dissertation project) has been completed by TGen Institute.

Secondly, the analysis investigating the effect of pre-diagnostic alcohol consumption on mortality among 1,286 female breast cancer cases has been completed and submitted to the journal, Cancer Epidemiology Biomarkers and Prevention. Briefly, we observed that women who consumed alcohol in the 5 years prior to diagnosis had a decreased risk of death [>0 to <3 drinks per week: HR(hazard ratio) = 0.7 (95% CI: 0.6-0.95); 3 to <7 drinks per week: RR = 0.6 (95% CI: 0.4-0.8); ≥ 7 drinks per week: RR = 0.7 (95% CI: 0.5-0.9)] compared to non-drinkers. Additionally, an analysis investigating the effect of pre-diagnostic smoking indicated no increased risk of death among breast cancer cases

Thirdly, an analysis investigating the potential for BRCA1 and BRCA2 germline mutations to alter the impact of chemotherapy and Tamoxifen on the risk of asynchronous, contralateral breast cancer (CBC) within 708 women with CBC and 1,399 women with unilateral breast cancer has been completed and a manuscript emanating from this work is currently under co-author review. Our findings indicated that the risk of CBC associated with chemotherapy and Tamoxifen did not differ between BRCA1/2 mutation carriers and non-carriers, except perhaps within certain chemotherapy regimens. Chemotherapy was found to reduce the risk of CBC in non-carriers (relative risk [RR] = 0.6 [95% CI: 0.5-0.7]) and carriers (RR = 0.5 [95% CI: 0.2-0.97]).

Also, an exploration into potential causes of inflammatory breast cancer (IBC) was determined to yield insufficient numbers of cases of this rare disease so that an analysis was deemed to be underpowered to detect risk factors specific to this breast cancer subtype.

Finally, data analysis for my dissertation project has been completed. For part 1 of my dissertation, I investigated the effect of genetic variation in the catechol estrogen metabolism pathway on breast cancer risk. We observed a statistically significant gene-gene interaction within the CYP1B1 and GSTP1 genes and breast cancer, such that women with 1 high risk genotype were at a 1.6-fold increased risk of breast cancer (95% CI: 1.03 – 2.4) , and women carrying 2 high risk genotypes were at a 2.8-fold increased risk (95% CI: 1.5-5.3) compared to women with 0 high risk alleles (test for trend: p-value = 0.03). Furthermore, we observed the breast cancer risk associated with carrying 1-2 high risk genotypes to be particularly strong for long-term combined hormone therapy (CHT) users (compared to users with 0 high risk genotypes, women with ≥1 high risk genotypes: OR = 3.3 [95% CI 1.02-10.4] among long-term CHT users; OR = 1.9 [95% CI: 0.4-9.3] among short-term CHT users; OR = 1.3 [95% CI 0.8-2.1] among never CHT. These data suggest that the risk of breast cancer associated with CHT use is modified by genetic variation in the catechol estrogen pathway. The manuscript detailing our findings is currently under review by members of my dissertation committee.

For part 2 of my dissertation investigating the effect of variation within 7 genes of the progesterone pathway, data analysis is complete and the manuscript is under preparation. Within this project, we observed breast cancer risk to be modestly associated with one SNP in each of 2 genes: *SRD5A1* (rs248793: OR=1.2 [95% CI: 1.02-1.5] for G homozygotes) and *PGR* (rs492457: OR=1.5 [95% CI: 1.01-2.1] for carriers of the A allele). We observed breast cancer risk related to each of these variants to be particularly heightened in long-term CHT users (rs248793: OR = 3.0 [95% CI: 1.6-5.7]; rs492457: OR = 2.0 [95% CI: 0.7-5.7]). However, we did not detect statistically significant gene-gene interactions within this pathway. Additionally, with the functionality of these 2 SNPs being unknown, it is less clear how the SNPs may interact with CHT to affect breast cancer risk, although our results suggest that breast cancer risk among CHT users is modestly modified by variation in these genes of the progesterone metabolism and receptor pathway.

Additionally, as part of the work with genes in the hormonal pathway, we are also exploring the potential for rare mutations within the *PGR* gene to be associated with breast cancer. Using long-range PCR techniques to sequence exons 1 and 2 of *PGR*, and a Solexa chip from Illumina which provides deep coverage of this sequence, we are investigating the association between rare polymorphisms and breast cancer within the same population of 2,351 women from my dissertation. If this exploration provides candidate polymorphisms, we could investigate whether any of these polymorphisms modify the effect of CHT within this population.

In addition to work specifically outlined in the SOW, but as put forth by the reviewers of the original grant application, I have continued to meet with Chris Carlson, a statistical geneticist, to discuss data analysis issues and strategies related to the large amount of data created by genotyping 71 SNPs in 2,351 women. In addition, I have attended multiple seminars specifically related to breast cancer research, including the Hormonal Chemoprevention of Breast Cancer and the Breast Cancer Prevention Forum at the AACR 2007 Annual Meeting, and multiple seminars at the FHCRC Breast Cancer Surveillance Consortium Symposium. Overall, I have made substantial progress towards the completion of my doctoral degree by completing the tasks set forth in year 2 of the Breast Cancer Research Program Pre-doctoral training grant, and I am well positioned to complete the remaining tasks in the final year of this grant.

Key Research Accomplishments

1. Completed the genotyping project consisting of 71 SNPs in 7 genes in 1,055 controls and 1,296 cases of breast cancer.
2. Investigated the extent to which BRCA1/2 germline mutations modified the effect of chemotherapy and Tamoxifen on the risk of asynchronous CBC
3. Submitted the manuscript describing the effect among breast cancer cases of pre-diagnostic alcohol consumption on mortality
4. Completed data analysis investigating genetic variation in the catechol estrogen pathway and breast cancer risk (part 1 of my dissertation)
5. Completed data analysis investigating genetic variation in tagSNPs and functional SNPs within the progesterone pathway and breast cancer risk (part 2 of my dissertation)

Reportable Outcomes

Manuscript, submitted: 'The Effect of Pre-Diagnostic Alcohol Consumption on Survival After Breast Cancer in Young Women,' Kerryn W. Reding, Janet R. Daling, Cecilia A. O'Brien, David R. Doody, Peggy L. Porter, and Kathleen E. Malone

Manuscript, currently being reviewed by co-authors: 'Adjuvant systemic therapy for breast cancer and the risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers,' Kerryn W. Reding, Jonine Bernstein, Bryan Langholz, Anne Reiner, and Kathleen Malone

Contribution to Poster Presentation, UW Teaching and Learning Symposium 2008: 'Innovative Teaching Methods for a Large Introductory Epidemiology Course,' Yuzo Arima, Kathryn Adeney, Zoe Edelstein, Sara Nelson, Amy Poel, Kerryn Reding, Britton Trabert, and Jack Goldberg.

Conclusion

In conclusion, the work completed this year has provided insight into potential mechanisms driving breast carcinogenesis, ranging from genes in the hormonal pathways affecting the impact of CHT on breast cancer risk to the effect of BRCA1/2 mutations on adjuvant therapy in breast cancer to the effect of alcohol consumption on survival in breast cancer.

References

None.

Appendix

Table 1. Statement of Work

TASK	STATUS
Task 1: Preparation for Lab Work (Months 1-4)	
a. Obtain Institutional Review Board approval	Previously Completed
b. Identify and prepare blood samples for DNA extraction (sample size (n) =2362)	Previously Completed
i. place samples in random order, intermixing cases and controls along with 10% quality control samples	Previously Completed
c. Coordinate the delivery/shipping of extracted DNA to CEEH and TGen	Previously Completed
d. Identify tagSNPs for AKR1C1 based on resequencing data (n = 24)	Previously Completed
e. Choose tagSNPs for AKR1C2, AKR1C3, SRD5A1, SRD5A2, PGR (SNPs already chosen for CYP1B1, COMT, and GSTs)	Previously Completed
Task 2: Coursework and Training-related Work (Months 1-12)	
a. Complete courses:	
i. Gene Structure and Function	Completed; modified task[1]
ii. Advanced Genetics of Human Diseases	Previously Completed
iii. Statistical Methods in Genetic Epidemiology	Previously Completed
iv. Teaching and Mentoring	Previously Completed
b. Prepare additional grants to support dissertation research	Previously Completed
c. Conduct data analysis on existing breast cancer data	Ongoing
d. Serve as Lead teaching assistant for Introduction to Epidemiology	Previously Completed
e. Conduct research on active learning techniques in Introduction to Epidemiology	Completed

Task 3: Project Oversight of Genotyping of Samples (Months 5-24)	
a. Monitor progress of assay development and implementation	Completed
b. Perform data management and project oversight	Completed
c. Perform independent quality assurance of 10% of samples at FHCRC (n = 237)	Modification request in progress[3]
d. Apply for and obtain IRB renewal	Completed for the remainder of the grant period.
Task 4: Training-related Work (Months 13-36)	
a. Present research findings on active learning at the UW Scholarship of Teaching and Learning Symposium	Previously Completed
b. Conduct data analysis on existing data related to breast cancer etiology	Ongoing task[2]
c. Serve as a teaching assistant for Introduction to Genetics	Previously Completed
Task 5: Data Analysis and Report Writing (Months 25-36)	
a. Perform data cleaning and coding of variables	Completed
b. Perform statistical analysis for each Specific Aim	Completed
i. Impute haplotypes using PHASE v.2 software	Completed
ii. Using STATA v.8, perform logistic regression analysis	Completed
iii. Using STATA v.8, perform polytomous regression analysis	Completed
c. Prepare manuscripts	Ongoing task
d. Present results at DOD Era of Hope conference	Planned for June 2008

[1] Substituted auditing the Biostatistics course, Statistical Evaluation of Biomarkers.

[2] Analysis investigating the effect of BRCA1/2 germline mutations on adjuvant therapies in asynchronous, contralateral breast cancers.

3 Modification involves performing a pilot investigation of rare SNPs in the first 2 exons of the PGR gene using remaining funds from the R03 project because quality controls were performed by the laboratory.